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Mutant IDH1 Dysregulates the Differentiation of Mesenchymal Stem Cells in Association with Gene-Specific Histone Modifications to Cartilage- and Bone-Related Genes(Abstract_要旨)

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論文題目	Mutant IDH1 Dysregulates the Differentiation of Mesenchymal Stem Cells in Association with Gene-Specific Histone Modifications to Cartilage- and Bone-Related Genes (変異型 IDH1 は遺伝子特異的なヒストン修飾を介して、間葉系幹細胞から軟骨及び骨への分化を脱制御する)		
(論文内容の要旨)			
<p>Enchondromas are benign bone tumors developing from intramedullary space and characterized by chondrocyte-like cell morphology and the production of cartilage matrix. Central chondrosarcomas are malignant counterparts of enchondromas and share these characteristics with them. Based on these pathological findings, cells that reside in bone marrow and have chondrogenic differentiation properties may be cell-of-origin of these tumors, and therefore mesenchymal stem cells (MSCs) among bone marrow stromal cells, which have differentiation properties for osteo-, adipo-, and chondrogenic lineages, are reasonable candidates as the precursor cells of these tumors. Although precise oncogenic mechanisms of these tumors are not yet known, somatic mutations of isocitrate dehydrogenase (<i>IDH</i>) genes have been found as driver mutations of these tumors. <i>IDH</i>s are metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG), and consist of a gene family with three members: <i>IDH1</i>, <i>IDH2</i> and <i>IDH3</i>, the first of which localizes in the cytoplasm while the latter two localize in mitochondria. Missense mutations of <i>IDH1/2</i> genes endow encoding proteins with neomorphic activity to produce the potential oncometabolite, 2-hydroxyglutarate (2-HG), which induces the hypermethylation of histones and DNA. <i>IDH</i> mutations were found first in gliomas, and subsequent analyses revealed that frequent and tumor type-specific mutations in enchondromas and central chondrosarcoma among various mesenchymal tumors. To know the role of <i>IDH</i> mutations in the development of these tumors, mutant <i>IDH</i> genes were introduced into human bone marrow-derived MSCs (hMSCs), and the biological effects were investigated.</p> <p>At first, mutation spectrums of <i>IDH</i> genes in Japanese samples were analyzed. Mutation of either <i>IDH1</i> or <i>IDH2</i> genes were found in 15/23 enchondromas and 20/54 chondrosarcomas, but no mutations were found in 7 osteochondromas or 29 osteosarcomas. This spectrum was equivalent with those reported previously. Because <i>IDH1</i> R132C was the most prevalent mutant in these tumors, subsequent analyses were performed using this type of mutant. The <i>IDH1</i> R132C gene was introduced into hMSCs by lentiviral infection, and the GC-MS analysis confirmed the production of 2-HG in cells stably expressing this mutant. Although no obvious changes were observed in cell morphology or proliferation property, the total amount of both active (H3K4me3) and repressive (H3K9me3 and H3K27me3) marks of histone methylation were increased, suggesting introduced <i>IDH1</i> R132C exerted its function to produce 2-HG and subsequently induced global histone methylation. Then the differentiation properties of hMSCs expressing <i>IDH1</i> R132C were investigated. Surprisingly, without the induction of chondrogenic differentiation, the expression levels of the <i>SOX9</i> gene, a master transcription factor for chondrogenesis, and <i>COL2A1</i> and <i>COL10A1</i> genes, major cartilage matrix components, were up-regulated in hMSCs expressing <i>IDH1</i> R132C, suggesting <i>IDH1</i> R132C promoted the chondrogenic differentiation of hMSCs. However, hMSCs expressing <i>IDH1</i> R132C failed to form tight cartilaginous pellets after 3-dimensional chondrogenic differentiation. As for osteogenic lineage-related genes, the expression of <i>RUNX2</i> and <i>Osterix</i> genes was up-regulated in hMSCs by the introduction of <i>IDH1</i> R132C. However, the expression of <i>alkaline phosphatase</i>, <i>liver/bone/kidney</i> (<i>ALPL</i>) gene was markedly down-regulated in hMSC expressing <i>IDH1</i> R132C. <i>ALPL</i> is a key factor in the mineralization, and consistent with the reduced expression of this gene, hMSCs expressing <i>IDH1</i> R132C failed to form calcified</p>			

nodules stained with Alizarin red and deposited markedly lower amounts of Ca after osteogenic differentiation, suggesting that *IDH1* R132C inhibited the osteogenic properties of hMSCs. This inhibitory effect was also observed in an osteosarcoma cell line with the inducible *IDH1* R132C gene.

To investigate whether these effects of *IDH1* R132C on differentiation were related to its effects on histone methylation of lineage-related genes, modifications of histones associated with the regulatory regions were investigated by chromatin immunoprecipitation (ChIP) assay. hMSCs expressing *IDH1* R132C showed increased amount of active mark associated with the regulatory regions of *SOX9* and *COL2A1* genes, whereas no significant increase was observed in repressive marks. In contrast, only the repressive mark (H3K9me3) was increased in the promoter region of *ALPL* gene in hMSCs expressing *IDH1* R132C. These results indicated that *IDH1* R132C dysregulates the differentiation properties of MSCs via gene-specific epigenetic modification, which may contribute to the tumor type-specific prevalence of *IDH* mutations.

(論文審査の結果の要旨)

軟骨形成腫瘍である内軟骨腫と軟骨肉腫は、ともに Isocitrate Dehydrogenase (IDH) の変異を有する。変異 IDH は 2-Hydroxylyglutarate を産生し、ヒストン脱メチル化酵素を阻害する作用等により癌化に寄与するとされているが、なぜ骨腫瘍の中で軟骨形成性腫瘍に特化して存在するのかは不明である。そこで本研究では間葉系幹細胞 (MSC) の分化能に対する変異 IDH の作用を解析した。まず骨腫瘍 113 例において、IDH1/2 遺伝子の変異を解析した。その結果、内軟骨腫 15 例（65%）、軟骨肉腫 20 例（37%）で変異が確認され、最も多い変異は IDH1 遺伝子の R132C 変異であった。一方、骨軟骨腫及び骨肉腫では変異は検出されず、既報と同等の結果が得られた。次にレトロウィルスを用いて R132C 型 IDH1 遺伝子を MSC に導入して、軟骨分化能を解析したところ、導入のみで SOX9 等の軟骨関連遺伝子の発現が亢進したが、3 次元分化誘導法を用いた評価では、軟骨塊が形成されず基質形成が阻害されている可能性が示唆された。一方、骨分化に関しては、RUNX2 等の骨関連遺伝子の発現は亢進したが、ALPL 遺伝子の発現が抑制され、Ca 沈着や石灰化結節形成能は著しく低下し、変異 IDH が骨形成を抑制することが示唆された。それぞれの遺伝子のヒストンメチル化を解析したところ、発現が亢進した SOX9 では active mark である H3K4me 3 が増加し、低下した ALPL 遺伝子では inactive mark である H3K9me3 が増加しており、変異 IDH がヒストンメチル化を介して分化能を制御していることを示唆する結果が得られた。

以上の結果は、軟骨形成性腫瘍における変異 IDH の役割の解明に貢献し、腫瘍発生機構を理解することに寄与することが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成 28 年 4 月 26 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。